PATENT
Docket No. 0825-0166P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Henrik GAROFF and Peter LILJESTROM Conf. No: 8395

APPLN. NO.: 09/901,106 GROUP: 1636

FILED: August 10, 1999 EXAMINER: Guzo, David

FOR: DNA EXPRESSION SYSTEMS BASED ON ALPHAVIRUSES

#12/E
Tuta
3/27/03

SUPPLEMENTAL AMENDMENT

Assistant Commissioner of Patents
Washington, DC 20231

December 19, 2002

Sir:

The following Supplemental Amendments and remarks are respectfully submitted in connection with the above-identified application in reply to the Office Action mailed August 23, 2002.

In the Claims:

Please add the following new claims:

--64. A helper cell for producing an infectious, defective alphavirus particle, comprising an alphavirus-permissive cell transfected with RNAs comprising:

(a) an alphavirus replicon RNA, wherein the replicon RNA comprises the alphavirus packaging signal and a heterologous RNA sequence, wherein the replicon RNA

furthermore lacks sequences encoding alphavirus structural proteins; and

(b) at least a first and second helper RNAs separate from said replicon RNA and separate from each other, said first and second helper RNAs encoding the structural proteins absent from the replicon RNA;

with said first helper RNA encoding at least one alphavirus structural protein and not encoding at least one other alphavirus structural protein;

with said second helper RNA not encoding said at least one alphavirus structural protein encoded by said first helper RNA and encoding said at least one other alphavirus structural protein not encoded by said first helper RNA;

and with said first and second helper RNAs lacking the alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNAs produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of the helper RNAs due to the absence of the structural protein coding sequences in the packaged replicon.

65. The helper cell according to claim 64, wherein said first helper RNA encodes both the alphavirus E1 glycoprotein and the alphavirus E2 glycoprotein, and wherein said second helper RNA encodes the alphavirus capsid protein.

66. The helper cell according to claim 64, wherein said first helper RNA and said second helper RNA both include a promoter.

67. The helper cell according to claim 64, wherein said replicon RNA includes a promoter.

68. The helper cell according to claim 64, wherein said inserted heterologous RNA is selected from the group consisting of RNA encoding proteins and RNA encoding peptides.

69. A method of making infectious, defective, alphavirus particles, comprising:

providing a helper cell according to claim 63;

producing said alphavirus particles in said helper cell; and then

collecting said alphavirus particles from said cell.

70. The method according to claim 69, wherein said alphavirus replicon RNA and said at least first and second helper RNAs are introduced into said helper cell by electroporation.--